

## 12.

The Morphology of the Pituitary Gland of the South African Clawed Toad, *Xenopus laevis* Daudin.

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(Plate I).

## INTRODUCTION.

In recent years much interest has been shown in the South African clawed toad, *Xenopus laevis* Daudin, because of its sensitivity to anterior pituitary hormones. On the basis of this sensitivity, Bellerby ('33) suggested *Xenopus* as a test animal for determining pregnancy. Landgrebe ('39) made such tests and described the technique in detail. The validity of the test was further evaluated by Weisman, Snyder and Coates ('42) and Weisman and Coates ('44). Hogben, Charles and Slome ('31), Bellerby and Hogben ('38) and Berk ('39) described sex factors related to pituitary function in this animal. During a somewhat earlier period a hypophyseal-chromatophore mechanism also received considerable attention (Slome and Hogben, '28).

Thus far while there appears to be agreement on the physiological aspects of the hypophysis of *Xenopus*, the morphological descriptions of the gland to be found in the literature (Rimer, '31; Hogben, Charles and Slome, '31; Levenstein and Charipper, '39; Atwell, '41) seem to be rather incomplete. The purpose of the present report is to describe the normal morphology and histology of the pituitary gland in *Xenopus laevis*, in an effort to augment and clarify the subject.

We wish to acknowledge with sincere appreciation the opportunity to examine the original preparations made by Dr. Irving Levenstein, in collaboration with the senior author during a preliminary survey, also the cooperation of Mr. Christopher W. Coates who through the New York Zoological Society and the New York Biologic Research Foundation generously contributed much of the material used in the current investigation.

## MATERIALS AND METHODS.

Sexually mature males and females of *Xenopus laevis* were used in this investigation. The pituitary glands were obtained following decapitation, and, usually, removed from the brain case along with a portion of the midbrain.

The tissues were fixed in Bouin, Zenker,

Formol sublimate, and formalin (10%) for studies of the microscopic anatomy of the gland. To demonstrate the finer cellular details, the tissue was placed in Champy's fluid for twenty-four hours, then in 2% osmic acid at 35° C. for three to six days. The Golgi apparatus was best demonstrated after the longer period of osmication.

Horizontal, longitudinal and cross-sections were then cut serially in paraffin at 3 and 4 micra. These were stained by Masson method with modifications (Foote, '33) and checked with hematoxylin and eosin. The Dawson and Friedgood ('38) method and the Severinghaus ('32) technique were used for further cellular differentiation.

## DESCRIPTION.

## a. Gross Morphology.

*In situ*, the pituitary gland when observed ventrally is a flattened disc-like structure with lateral elongated projections. The anterior portion of the gland is bounded ventrally by the presphenoid cartilage which extends forward from the basisphenoid bone. When these structures are removed, the gland is seen to be suspended from the *tuberculum cinereum* by the infundibulum just dorsal to the optic chiasma (Fig. 1).

The *pars anterior* is ventro-caudal to the rest of the gland. In some specimens this portion is ovoid in outline with its longer dimensions antero-posterior while other specimens exhibit a greater diameter in the dextri-ventro axis. Dorsal to this ovoid structure is the flattened, elongated, dumb-bell shaped extensions of the *pars intermedia*, which extend transversely across this anterior lobe. No large blood vessels are observed entering the pituitary body, but a good sized blood vessel is seen embedded in both sides of the brain, lateral to these elongate projections.

## b. Microscopic Anatomy.

## 1. General.

A histological study of the *hypophysis cerebri* of *Xenopus laevis* reveals a structure, similar, in general, to that described for most vertebrates. In serial sections, and at the

several planes, this gland shows an anterior portion with a cellularly differentiated area (the anterior process of Atwell '41), a *pars intermedia*, a *pars nervosa* and, lying on either side of the median line, two epithelial plaques embedded in the walls of the infundibulum—the *pars tuberalis*.

The *pars anterior* is ventro-caudal to the rest of the gland (Fig. 2) and is ovoid in shape. It is the largest lobe of the complex and is firmly embedded in a thickened median eminence of the infundibulum. The rostral area of the *pars anterior* is heavily vascularized and distinct from the anterior lobe proper. The area consists chiefly of weak staining basophile cells with few acidophiles.

Dorsal to the anterior lobe is a flattened elongate structure which extends transversely across the anterior lobe (Fig. 3). These projections consist of two clearly demarcated areas, a ventral portion, the *pars intermedia* and a dorsal portion, the *pars nervosa*. The intermedia is very closely applied to the *pars nervosa* and only separated by a thin connective tissue septa. The *pars nervosa* is continuous with the infundibulum.

Anterior to and continuous with the infundibulum on either side are thickened extensions of the hypothalamus, the *tuber cinereum*. These structures completely enclose the infundibular cavity which, in some sections, is continuous with the third ventricle of the brain.

In transverse and frontal sections of the *tuber cinereum*, two epithelial plaques (*pars tuberalis* of Atwell '41), one on either side, are found embedded in the ventral surface (Fig. 5). These structures are bounded on the ventral surface by the *pia mater* and on the dorsal side by blood vessels.

## 2. Pars Anterior.

Of the several structures which make up the pituitary gland, the *pars anterior* is the most complex in its cellular elements. It is seen as nests in transverse sections. These nests are separated by delicate connective tissue partitions (Fig. 4). Very often a capillary can be seen penetrating the center of the nest of cells. In median sagittal sections, the gland is somewhat tubular and consists of cords of cells which are separated by capillary channels containing numerous erythrocytes.

The *pars anterior* fixed in Bouin's, Champy's, or formalin and stained in Masson's stain consists of three general cell types, acidophiles, basophiles and chromophobes. These cells can be distinguished by size, granulation, and staining reaction.

With the Dawson-Friedgood technique ('38), it is possible in some sets of slides to distinguish four cell types on the basis of staining reactions. Two types of acidophiles may be distinguished; an "Azure-carmine" cell and an "Orange G" cell. The "Azure-carmine" cell is larger than the "Orange G," and

contains granules which are coarser and more numerous than those present in the "Orange G" cell. The cytoplasm of the "Orange G" cell is homogenous in appearance, containing very fine acidophilic granules evenly distributed throughout the cell. The nuclei of both of these cells are identical; ovoid with some chromatin material and a very definite acidophilic nucleolus. The third cell type is a deeply staining basophile; the fourth cell type is very much like the "Orange G" cell in size and cytoplasmic granulation, but is distinguishable by lightly staining purple cytoplasm. These cells are sparse.

The distribution and staining intensity of the cells is not uniform throughout. The periphery of the gland appears to contain poorer staining cells. In the rostral portion of the anterior pituitary, below the anterior process, only a very few deeply stained acidophilic cells occur. It was noted, however, that acidophilic cells become more numerous near the center of the gland and diminish toward the periphery. Few well stained basophile cells are encountered in the anterior portion of the gland, but many occur in nests deeper in the anterior pituitary, a condition very much similar to that described for the pituitary gland of *Necturus maculosa* (Charipper, '31).

The acidophile cells are completely filled with coarse spherical granules, and show definite cell outlines (Fig. 10). They contain an ovoid vesicular nucleus within which is a central acidophilic nucleolus. When exposed to osmic acid, the Golgi apparatus appears as a network capping the nucleus.

The basophile cells, though relatively few in the anterior lobe, show a tendency to group and form nests. The granules in the basophile are not as coarse or as numerous as those found in the acidophiles. These cells when exposed to osmic acid demonstrate many clear vacuoles within the cell. The nucleus is not as large as that of the acidophile and is indented or somewhat kidney-shaped. This nucleus is usually clear and contains a definite acidophilic nucleolus. The typical Golgi found here is similar to that described by Severinghaus ('33) for the pituitary of the rat. It occurs in the cytoplasm away from the nucleus. The Golgi network does not appear as a continuous structure but rather as though made up of heavy plates (Fig. 8).

The third type of cell, the chromophobe, in osmicated preparations is light brown in color and takes neither the acidophilic or basophilic stain even after post-chromatization. This cell is more like the acidophile than the basophile in shape, and is found distributed throughout the gland but is the dominant cell in the rostral part of the anterior pituitary. The cytoplasm of these cells is finely granular. The nucleus is vesicular and contains a central nucleolus similar to those found in acidophiles and basophiles. In some

of these cells, Golgi of the acidophilic cell type is found. In others, Golgi characteristic of the basophile cell occurs.

### 3. Anterior Process.

The terminology adopted here is that suggested by Atwell ('41) although this structure was described by Rimer ('31) and Hogben and Slome ('31) as the *pars tuberalis*.

The anterior process is embedded in the median eminence of the infundibulum. This area, which is continuous with the *pars anterior*, is the point of attachment of the anterior lobe with the infundibulum. The cells of the anterior process are arranged in very definite cords, giving this area a lobular appearance. In transverse sections, these cords appear as nests, each of which is completely within a thin connective tissue septa (Fig. 6). A small lumen may be seen in the center of each nest. Each of these acinar-like structures contains two types of cells. One of these cell types is similar to that found in the *pars tuberalis*. It is chromophobic with a vesicular nucleus and a central acidophilic nucleolus. The cells are ovoid in outline with very little cytoplasm and show poor cell boundaries. The second cell type found within these nests is basophilic, columnar, and contains a nucleus similar to that found in the chromophobe. This basophilic cell is larger than the chromophobe and the nucleus is always basally placed.

The anterior process is heavily invaded by blood vessels from the infundibulum. These vessels penetrate the anterior process and make their way to the anterior lobe proper.

### 4. Pars Tuberalis.

The center of each plaque forming this part of the gland is pierced by fair sized blood vessels and is well vascularized (Fig. 7). The cells of the *pars tuberalis* in some sections appear to be arranged in cords and separated by thin connective tissue septa (Fig. 9). This cord-like arrangement, however, is not as clear as that found in the anterior processes. The cells of the *pars tuberalis* are quite different from those found elsewhere in the gland. They are comparatively small and the cells are chromophobic with scant cytoplasm. The nucleus which is vesicular occupies the greater portion of the cell. Unlike the cell membrane, the nuclear membrane is well defined. Within the clear nucleoplasm is a large, distinct, usually centrally placed, acidophilic nucleolus.

### 5. Pars Intermedia.

The *pars intermedia*, as seen in transverse section, is situated between the *pars anterior* and the *pars nervosa*. In frontal section, this portion of the gland is thickest at either lateral end, but only three to four cells in width where the *pars intermedia* forms a concavity into which the oval region of the *pars anterior* fits. The *pars intermedia* is characterized

by its compact irregular cord-like arrangement. These cords are separated from each other by thin connective tissue. This lobe of the pituitary is separated from the *pars anterior* and the *pars nervosa* by well defined connective tissue partitions. No evidence of direct vascularization could be found.

The cells making up the cords of the *pars intermedia* are very tall, columnar, with small basal, ovoid nuclei. When stained with aniline blue after routine fixation, these cells react as basophiles. In cross section the cells are ovoid and have poor cellular outline. The nucleoplasm stains weakly with acid fuchsin but contains a deep staining acidophilic nucleolus. The cells of the *pars intermedia* are very rich in osmophilic material (Fig. 11). Some Golgi configurations such as described for the acidophiles and basophiles of the *pars anterior* are present, but more often the osmophilic material is found either as small rings or thick short filaments, distributed throughout the cell.

### 6. Pars Nervosa.

This portion of the gland lies dorsal and adjacent to the *pars intermedia* and is separated from the latter only by a connective tissue membrane. The *pars nervosa* is continuous with the infundibulum and consists almost entirely of rather compact basophilic fibrous tissue. These fibres are arranged in cords giving the *pars nervosa* a lobular appearance. Distributed throughout this fibrous mass are several different cellular elements. Especially abundant are the ependymal cells similar to those which line the anterior wall of the infundibulum and the *tuber cinereum*. Mossy neuroglia cells also are present with a scattering of some spindle-shaped cells with a process at each end of their long axis. Many basophilic cells resembling those of the *pars intermedia* can be distinguished here. The *pars nervosa* is well vascularized and contains many blood sinuses and capillaries. The presence of these sinuses give the *pars nervosa* a very sacculated appearance. These blood vessels enter the *pars nervosa* at the region where this portion of the pituitary comes off the infundibulum.

### DISCUSSION.

The pituitary gland of *Xenopus laevis* Daudin, in relation to its neurocranium, is similar to that found among the anurans. The entire gland is suspended from the hypothalamus and although when exposed on its ventral surface it is easily movable, the anterior lobe—unlike that of other anurans—is firmly attached to the brain. No major blood vessels penetrate the gland although even macroscopically the anterior lobe is seen to be richly vascular. This concurs with the report of Patterson ('39), who described the vascularization of the brain of *Xenopus* as being similar to that of urodeles and other anurans.

The ramus posterior, a branch of the internal carotid arteries, divides and one of its branches is distributed to the optic lobe while the other vascularizes the pituitary body. Blood is carried to the jugular vein from the pituitary by two very thin-walled veins. These connect with each other by transverse vessels. In the anurans these thin-walled veins are described by Ecker (1889) as small venous plexi on either side of the pituitary body.

The general structure of the pituitary of *Xenopus* is similar to that described by other investigators (Atwell '19, '41; DeBeer, '26; Charipper '31; Rimer '31; and Sato '35). The four characteristic lobes found in all amphibians are present in this animal. As pointed out by Charipper ('37), the pituitary body of amphibians is not compact. This is especially true of *Xenopus* where two elongate lateral extensions are found projecting transversely across and beyond the ovoid anterior pituitary.

The *pars anterior* of *Xenopus laevis*, like that of other amphibians, is ventro-caudal to the rest of the gland. This is transversed on its dorsal side by the *pars intermedia* and *pars nervosa* which are dumb-bell shaped. The *pars tuberalis* in this animal is located in a position similar to that described by Atwell ('19) for *Rana pipiens* and *Rana catesbeiana*. This lobe is described by DeBeer ('26) as consisting of a pair of epithelial plaques "plastered" on the floor of the *tuber cinerum* in front of and separate from the rest of the pituitary body.

The anatomical relationships of these parts, therefore, do not vary from those described in other amphibians. In microscopic preparations, however, *Xenopus laevis* displays an area of cells not occurring in either urodeles or anurans. This particular area is found at the rostral portion of the *pars anterior* and is embedded in a thickened portion of the floor of the infundibulum which Atwell ('41) designates as the median eminence. The cells of this area are of two types, basophilic and chromophobic. The area is well vascularized. This portion of the anterior pituitary, because of its cellular arrangement, cell types and heavy vascularization, is suggestive of the *pars tuberalis* of urodeles described by Atwell ('21) and DeBeer ('26). On the basis of this description Rimer ('31) and Hogben, et al. ('31) have called this area the *pars tuberalis* and concluded that *Xenopus laevis* displayed a "zalamandrine" type of pituitary. As a further confirmation of this, Rimer ('31) reports that despite a careful study of serial sections he was not able to locate the *pars tuberalis* as described for anurans. Atwell ('41), in discussing the anterior margin of the *pars anterior*, is of the opinion that it is not the *pars tuberalis* but rather a differentiated area of the *pars anterior*. He describes the *pars tuberalis* of *Xenopus* as epithelial plaques in the ventral

wall of the infundibulum. Dawson ('40) also reports a similar region in the anterior margin of the *pars anterior* in the pituitary of the African lungfish. This region being single and median in position, does not fit any phylogenetic description of a true *pars tuberalis*.

In this work both the anterior margin of the *pars anterior* as well as the infundibulum were studied serially in transverse, sagittal and frontal planes. The cells were also stained with several techniques to demonstrate cellular detail. As a result, both the anterior process and the paired epithelial plaques as described by Atwell ('41) for *Xenopus laevis* were seen and confirmed. The study of the anterior process is in agreement with that described by Rimer ('31) and Hogben, et al. ('31) for this area. The cell types and arrangement of cells in cords is similar to that described by Atwell ('21) and DeBeer ('26) for the *pars tuberalis* of urodeles. The *pars tuberalis* in *Xenopus laevis* is located in a position similar to that described by Atwell ('19 and '41). However, the present findings differ in that this area showed some tendency to be lobular and definitely vascularized. This is in accord with Sato ('35) and D'Angelo ('41). The latter investigator suggests that the vascularization of the *pars tuberalis* in the frog is appreciable when measured in terms of capillary length.

Atwell ('41) points out that morphogenetic and cytologic studies are necessary to determine whether the anterior process represents the region of earlier attachment of the *pars tuberalis* and whether these two areas are related histologically. In this connection he describes the cells of the anterior process of *Xenopus* to be larger and more definitely chromophilic than those of the *tuberalis*. In this investigation, however, we are able to demonstrate many chromophobic cells within the cords of the anterior process which are identical with the cells of the *pars tuberalis*. However, no valid cytological criteria could be found for further confirmation of the relationship unless negative results be considered. Golgi material could not be demonstrated in the cells of the anterior process or in the cells of the *pars tuberalis*.

The tubular arrangement of the cells in the *pars anterior* of *Xenopus laevis* seems to be peculiar to this form since similar conditions are not described in the literature for other amphibians. Poris and Charipper ('38) report this condition common to all reptilian forms and an outstanding feature of the *Anolis carolinensis* pituitary. In amphibian pituitary, three types of cells are distinguished; acidophiles, basophiles and chromophobes. In their distribution the chromophobe cells are found in all parts of the *pars anterior* and are the dominant cell in its anterior portion. The acidophiles are scant in this area but are more numerous in the center of the gland. There are few true baso-

philes present in the anterior lobe and these are found only in its posterior region. A study of the osmophilic material in these cells reveals bizarre configurations. Many acidophile cell demonstrate Golgi apparatus capping the nucleus, similar to the acidophiles in the rat's pituitary (Severinghaus, '33). This configuration in *Xenopus laevis* is not constant for all acidophiles. Basophiles treated with osmic acid also displayed osmophilic material. The Golgi apparatus is usually away from the nucleus and ring-shaped. This cell, unlike the acidophile, contains many clear vacuoles. Among the chromophobes both types of Golgi configurations are found. This condition is similar to that described by Severinghaus ('33) for the rat and Levenstein ('39) for the pituitary of goldfish.

The *pars intermedia* of *Xenopus*, in general, conforms with that found in other amphibians. As described for most amphibians this lobe of the pituitary contains only one cell type which stains basophilically. Atwell ('19), however, maintains that the cell type of the intermedia is chromophobic and further that that part is well vascularized. The present investigation yields no evidence of direct vascularization in this lobe. This agrees with the work of DeBeer ('26) and D'Angelo ('41) for Anura. The cells of the intermedia are very rich in osmophilic material. Seldom, however, do these assume definite configurations but, rather, appear scattered as short rods throughout the cell.

The *pars nervosa* is continuous with the infundibulum and is composed chiefly of coarse basophilic fibres. This portion of the gland contains cords which give it a somewhat lobular appearance. The several cell types present here are chiefly nervous in origin. However, these cells are similar to those present in the *pars intermedia*. The *pars nervosa* is very conspicuously vascularized and contains many large blood sinuses. This is in general agreement with all such previous investigations.

#### SUMMARY AND CONCLUSIONS

1. Confirmation is offered for the presence of five anatomical parts of the pituitary gland in *Xenopus laevis* Daudin; *pars anterior*, anterior process, *pars intermedia*, bilateral plaque-like *pars tuberalis* and a *pars nervosa*.

2. The *pars tuberalis* in *Xenopus* is described as definitely vascular.

3. The *pars anterior* contains the usual three cell types: acidophiles, basophiles and chromophobes.

4. Two varieties of chromophobes are described on the basis of their Golgi configuration. One with a compact Golgi capping the nucleus similar to that described in the acidophile; the other with a looser, thinner, band-like arrangement not closely applied to the nucleus and similar to the condition found in

the basophile. This arrangement is very much like the configurations described by Severinghaus ('33) for the rat and Levenstein ('39) for the goldfish.

5. The cell types found in the anterior process, compared to those occurring in the *pars tuberalis*, indicate a possible histogenetic relationship and lend positive weight to Atwell's ('41) question concerning the anterior process as a point of attachment of the *pars tuberalis*.

6. The confirmation of the presence of the *pars tuberalis* as definitely bilateral epithelial plaques further strengthens Atwell's ('41) contention that the pituitary of *Xenopus* is definitely not of the "zalamandrine" type.

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## EXPLANATION OF THE PLATE

## PLATE I.

- FIG. 1. A ventral view of the pituitary gland of *Xenopus laevis* Daudin, in its relation to the brain. Note the ventrocaudal position of the anterior lobe and the lateral elongated projections dorsal to this structure. **A. L.**, Anterior lobe; **M. O.**, Medulla oblongata.
- FIG. 2. A median sagittal section of the pituitary showing the relationship of the *pars nervosa* (**P. N.**) *pars intermedia*, (**P. I.**) *pars anterior* (**P. A.**), and the anterior process (**A. P.**) in its relationship to the infundibulum. Formalin-sublimate; Dawson-Friedgood,  $\times 135$ .
- FIG. 3. Transverse section through the pituitary gland. Note the wing-like arrangement of the *pars intermedia* and the *pars nervosa*. Formalin; Mallory modification.  $\times 50$ .
- FIG. 4. Section through the *pars anterior* showing nesting arrangement, three types of cells and in addition the heavily stained connective tissue system. Bouin; Masson.  $\times 1080$ .
- FIG. 5. Anterior process (**A. P.**) embedded in this median eminence (**M. E.**). Note the epithelial plaque, the *pars tuberalis*, (**P. T.**) in the broad arm-like tuber cinerum (**T. C.**). Formalin; Mallory modification.  $\times 200$ .
- FIG. 6. Section showing two cell types present in the anterior process, the darker basophilic cells and the lighter chromophobic cells. Mann-Kopsch; Masson.  $\times 650$ .
- FIG. 7. A higher magnification of the *pars tuberalis* showing its vascularization. Formalin; Mallory modification.  $\times 650$ .
- FIG. 8. A highly magnified region of the *pars anterior* showing the loose Golgi network of a basophile. Mann-Kopsch; Masson.  $\times 1800$ .
- FIG. 9. Section through the epithelial plaque forming part of the *pars tuberalis*, showing the chromophobic nature of the cells and their striking similarity to the chromophobes of the anterior process drawn in Fig. 6. Formalin; Mallory modification.  $\times 1440$ .
- FIG. 10. Section through the *pars anterior* showing the vesicular nature of the nuclei. In addition the different Golgi configurations may be observed. Mann-Kopsch; Masson.  $\times 1440$ .
- FIG. 11. A section through the *pars intermedia* showing the amount and distribution of the osmophilic substance and to some extent the nature of the Golgi configuration. Osmo-sublimate; Dawson-Friedgood.  $\times 1800$ .